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FORMULATION AND STABILITY OF THE ANTINEOPLASTIC AGENT: N,N'-Di-9-ACRIDINYL-1,6-HEXANEDIAMINE (NSC #219713)

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ABSTRACT

N,N'-di-9-acridinyl-1,6-hexanediamine (NSC #219713) was solubilized and lyophilized for parenteral use in the form of the methanesulfonate salt. Stability studies of the drug in solution of pH 3.0, 4.5 and 7.0 and at both 25° and 70°C were carried out and the drug was stable at 25°. At 70°C slight degradation was observed in solutions of pH 3.0 and 4.5, while extensive loss occurred at pH 7.0 with a half-life of 16.5 hours. The lyophilized preparation was stable for one week when reconstituted with water and diluted with 5% dextrose solution. Precipitation of the drug as the hydrochloride salt occurred when dilution was done with normal saline solution. Stability was monitored using high performance liquid chromatography.

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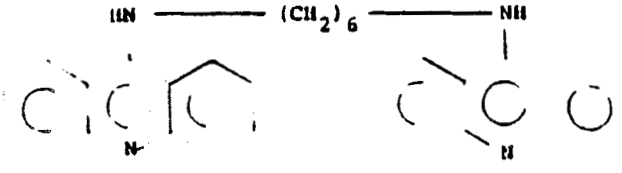
INTRODUCTION

Quinacrine has been used in the treatment of neoplastic effusions[1,2]. Certain acridines have selectively localized in tumor nuclei under in vivo conditions[3,4], intercalcating with nucleic acids[5,6]. Davis and coworkers[7], used acridine as a carrier to promote the localization of boron into tumors for neutron-capture therapy. Recently, nitrogen mustard containing acridines have shown antitumor activities[8-16].

Based on the classical studies of Lehrman[5,6] on the intercalation of proflavaine with DNA and the kinetic studies of Crothers and coworkers[17,18], Canellakis, et al. synthesized[19] and studied the antileukemic activities[20,21] of a homologeous series of diacridines. N,N'-di-9-acridinyl-1,6-hexanediammine(I) was one of this series



(I)

The present study deals with the development of an acceptable soluble and stable parenteral dosage form suitable for experimental evaluation of the anticancer activity of compound I.

RESULTS AND DISCUSSION

N,N'-di-9-acridinyl-1,6-hexanediamine was supplied in the base form which was of limited aqueous solubility (<< 0.1

mg/ml). For experimental evaluation of the potential chemotherapeutic utility of the drug, an intravenous solution was desired. In order to avoid the need for long term solution stability studies, formulation of lyophilized product was decided upon. In view of the fact that I is basic compound (pK_a 10), initial attempts were aimed at the identification of a suitable water soluble salt. The solubility of I was monitored as a function of pH in aqueous media containing various acids. The data obtained showed a maximum solubility in all cases at pH ~7.0. The maximum solubilities observed, the pH of maximum solubility, and the acids used are shown in Table I.

In view of this solubility data, a formulation containing 4 mg/ml of I (as the acetate or methanesulfonate salt) appeared to be reasonable. The acetate salt was prepared by dissolving the drug in excess acetic acid with heating. The solution was cooled and the salt was precipitated out of the solution by adding ether. The product (m.p. 157-158°C) when analyzed for C,H,N, indicated the presence of 4 moles of acetic acid per

Table I: Solubility of Compound I in Different Acids

Acid and pH	Solubility (mole/L)	Solubility Product
Hydrochloric acid (pH 6.7 - 7.0)	2.34×10^{-3}	1.28×10^{-8}
sulfuric acid (pH 6.96)	1.06×10^{-4}	1.12×10^{-8}
citric acid (pH 7.44)	1.28×10^{-4}	1.64×10^{-8}
acetic acid (pH 7;12)	1.17×10^{-2}	1.6×10^{-6}
Methanesulfonic acid (pH 7;12)	1.15×10^{-2}	1.52×10^{-6}

mole of the drug in the crystalline product. The non-aqueous determination of the equivalent weight of the salt agreed with the elemental analysis (calc. 355, found: 347, one determination). Compound I (4 mg/ml) was formulated with acetic acid by freeze drying and when the lyophilized samples were reconstituted with water, incomplete dissolution occurred, and the soluble phase exhibited pH ~ 8.2 suggesting that acetic acid was volatilized during the lyophilization process.

In order to avoid the volatilization problem, we turned to the stronger and less volatile, methanesulfonic acid. The methanesulfonate salt of the drug was prepared by the heating and I (0.5 g,1.1 mMole) with the calculated amount of the acid (7.3 ml of 2%, 2.2 mMole) in aqueous solution. The salt produced was difficult to isolate in a dry crystalline form. Therefore, subsequent work involved the formulation of the salt *in situ*. The drug (4 mg/ml) was added to aqueous methanesulfonic acid (in molar ratio of 1:2) containing 1% w/v mannitol. Aliquots (10 ml) of the resulting solution (pH 4.3) were frozen and lyophilized. Redissolution was rapid when water (10 ml) was added to the lyophilized product. Reconstituted samples when allowed to stand at ambient laboratory conditions, showed no precipitate formation over a three week period. When such solution was diluted and analyzed by HPLC, no degradation was detected to have occurred. These results indicated that this prototype formulation was promising and subsequent work was done using this formulation. Incorporation of mannitol (at 1% w/v) was indicated in the formulation as a bulk agent and to aid in the formulation of a rapidly dissolving porous lyophilized cake. When mannitol was detected, the lyophilized product was found to be sticky and reconstituted only slowly.

The HPLC method developed was suitable for monitoring both I and its degradation products (Figure 1) and the results for the analysis indicated the accuracy and the reliability of the chromatographic system.

The stability studies were concerned with the effect of light, pH, and temperature on the degradation of I. The stability of the drug was found not to be affected by exposure to or protection from light under the selected conditions of pH and temperature. In aqueous solution at 25°C and pH values of 3.0 (0.01 M methanesulfonate), 4.5 (0.1 M acetate) and 7.0 (0.01 M phosphate), no loss of the drug or appearance of de-

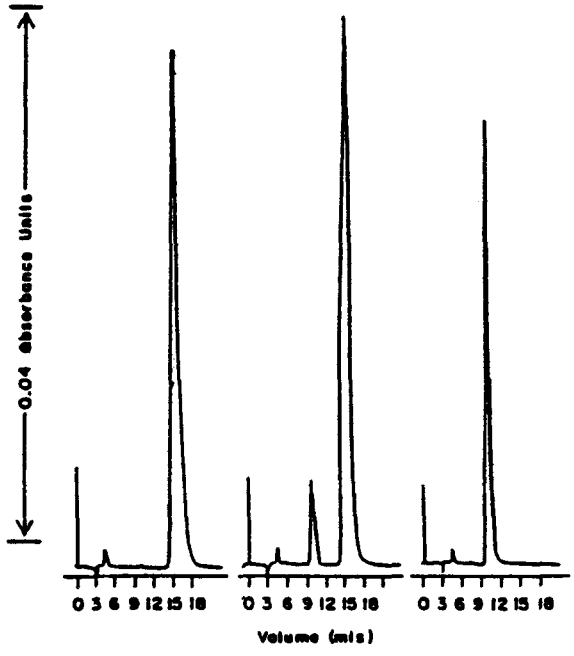


Figure 1. High Performance Liquid Chromatography of solutions of NSC 219733; a) freshly prepared solution (35 µg/ml), b) after 14 days at 70° (35 µg/ml) and pH 4.0, and c) after 14 days at 70° and pH 7.0 (15 µg/ml). Peak at ∿ 15 ml is drug and peak at ∿ 10 mls is degradation product.

gradation product was obtained over 7 days. However at 70°C, in the same solutions, some degradation was observed at pH 3.0 and 4.5 over a 7 day period (Figure 1b). At pH 7.0, loss of the drug (Figure 1c) occurred by an apparent first order process which exhibited a half-life of ~ 16.5 hours (Figure 2). When the lyophilized preparation (4 mg/ml) was reconstituted and maintained at 25°C, no degradation was observed by HPLC analysis over one week period.

Since many products for intravenous use are administered as infusion and are routinely diluted with parenteral vehicles, dilution (50 folds to give 0.08 mg/ml) of the reconstituted product with 5% dextrose and normal saline solutions were made. When the sample was diluted with 5% dextrose solution and stored

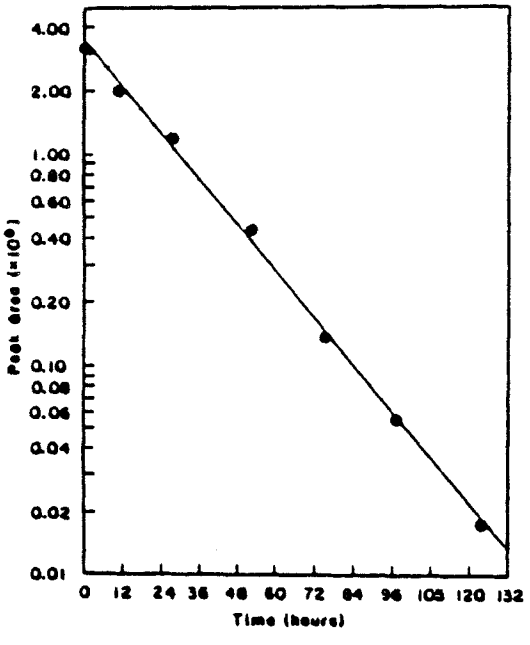


Figure 2. Semilog plot of peak area (corresponding to concentration) of NSC 219733 remaining as a function of time for a solution at 70° and pH 7.0.

at 25°C for one week, no degradation was detected. However, as the dilution was done with normal saline solution, a precipitate was formed at the point of mixing the two solutions. After vigorous shaking, redissolution appeared to occur, but after setting for one hour, a large amount of precipitate developed and it did not redissolve. When this precipitate was separated and analyzed, as expected, found to be the dihydrochloride salt of I. On the basis of the solubility of the dihydrochloride salt (Table I), the K_{sp} = (solubility)^3 ~ 1.28×10^{-8}. Thus in normal saline ($[Cl^-]$ = 0.15), the solubility of I would be expected to be only about 5.7×10^{-7} M. Consequently, dilution with saline is to be avoided. It is also worth considering whether or not intravenous administration of the reconstituted product may be risky due to the concentration of the chloride ion (0.1 M) present in the blood. Formation of a precipitate *in vivo* would be expected to be a function of the drug concentration of the administered solution, the rate of administration and the effect of other constituents of the blood, e.g., proteins, etc. to retard or prevent precipitation. Such factors are best evaluated by actual *in vivo* studies in animals.

In order to ascertain whether the product developed in this study will be ultimately clinically useful, the samples of the lyophilized product have been provided to the National Cancer Institute for animal studies.

EXPERIMENTAL

Reagents:

N,N'-di-9-acridinyl,1,6-hexanediamine (NSC #219733), was used as supplied by the Drug Development Branch, National Cancer

Institute. Methanesulfonic acid from Aldrich Chemical Company was used as obtained. All other chemicals were analytical or chromatographic grades.

Procedures:

Chromatography: High performance liquid chromatography was performed using a Waters Associates Model 204 system together with a Varian CPS-111C integrator. The system utilized a (Waters Associates) μ-Bondapak C_{18} column. The flow rate of the mobile phase (methanol:ammonium dihydrogen phosphate 0.15 M of pH 4.0 solution (1:1)) was 2 ml/minute. Analysis was carried out at ambient temperature, detection was at 254 nm at a sensitivity of 0.05 AUFS, and the volume of the solution injected was 10 μl. All quantitative measurements were based on measurements of peak area.

A stock solution for the standard curve was prepared by dissolving compound I (10 mg) in 0.01 N hydrochloric acid (200 ml). This solution was diluted as necessary with the same solvent to yield the various concentrations required for constructing the standard curve. The peak area of compound I was plotted vs the corresponding concentration to give a linear relationship (regression analysis gave a slope of 2.099×10^{8}, and intercept of 8×10^{-3} and a correlation coefficient of > 0.999).

Formulation of Compound I:

Water (358.5 ml) was added to compound I (1.5184 g, 3.227 mMole) and mannitol (3.8 g) and the mixture was treated with 2% (v/v) of methanesulfonic acid (21.48 ml, 6.454 mMole). The solution was kept in a sonic bath for 1 hour to aid dissolution, and the resulting solution was passed through a membrane filter

(type SS Millipore, 3.0 μ). The pH of the filtered solution was 4.30. Aliquots (10 ml) of the filtered solution were placed in 30 ml vials, flash frozen in a mixture of acetone and solid carbon dioxide and transferred to a freeze dryer in which the shelf temperature was -40°C. When the initial drying cycle was completed (shelf temperature reached zero), the samples were subjected to secondary drying for an additional 5 hours. The temperature was allowed to rise to ambient conditions and the vials were stoppered and sealed. Analysis of the lyophilized product involved addition of 10 mls of water to each vial to be analyzed. An aliquot was removed and diluted 100-fold with 0.01 N hydrochloric acid. A portion of this solution was then analyzed by HPLC for compound I.

Stability Studies of Compound I in Buffer Solutions of pH 3.0, 4.5 and 7.0 at 25° and 70°C

Compound I was dissolved in the selected buffer solution (1.5×10^{-2} mg/ml for pH 7.0, 3.5×10^{-2} mg/ml for pH 4.5 and 3.9×10^{-2} mg/ml for pH 3.0). The solutions obtained were divided into 2 groups with one being protected from light (by wrapping in foil). All samples were placed in a constant temperature bath at either 25°C or 70°C and samples were withdrawn and immediately frozen at various times over a period of one week. All samples were subsequently thawed and analyzed at the same time. The stability study of the reconstituted solution after dilution with dextrose solution. The stability of drug in dextrose solution involved reconstitution of a vial of lyophilized product with 10 ml water. An aliquot (2 ml) was diluted to 100 ml with 5% dextrose solution and the solution was maintained at 25° for one week.

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